

Case study: Tuberculation, serology and bacteriology of sows at a farrowing unit suspected of an infection with *Mycobacterium avium*.

Oorburg, D.^{1,5*}, Hiller, A.^{1,5}, Bakker, D.², Wisselink, H.², Buholzer, P.³, Cooremans, S.⁴, Heres, L.⁵, Urlings, H.A.P.^{1,5}

¹Wageningen University, Animal Sciences Group, Wageningen, the Netherlands

²Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands

³Prionics, Schlieren, Switzerland

⁴Lintjeshof, Nederweert, Netherlands

⁵VION Food Group, Boxtel, The Netherlands

* VION Food Group, P.O. Box 1, 5280 AA Boxtel e-mail Derk.Oorburg@vionfood.com

Abstract

Mycobacterium avium (MA) is considered a zoonotic hazard in pork. Herds delivered at slaughter showing gross lesions indicative of a mycobacterial infection, eg. specific abscesses in lymphoreticular tissue, were bacteriologically positive for MA.

A risk factor analysis revealing different possible sources of primary infection was carried out at farms supplying these pigs. Also the common farrowing farm supplying the piglets to these farms was taken into account as a possible source of infection.

Intradermal tuberculin testing, serology and tissue sampling was carried out on the sows and finishing pigs.

Positive results in tuberculation, serology and bacteriology of pigs and bacteriology of environmental samples are presented. Intradermal tuberculation below the tail is compared with the standard procedure of testing behind the ear (lege artis). This new method of tuberculation is easier to perform.

Using relevant slaughterhouse information can be an effective tool to improve the control of food safety hazards in the pork production chain.

Introduction

Mycobacterium avium (MA) is considered a zoonotic hazard in pork (1,2) and can cause severe illness in immune-compromised people and young children (3,4).

Known risk-factors for the infection of pigs with MA are feeding of contaminated peat, compost, sawdust/wood shavings and pests (5,6,7,8).

These infections can cause typical gross lesions eg. abscesses in lymphoreticular tissue (9). All slaughter pigs are subjected to post mortem meat inspection (PM) according to EU legislation (EU/854/2004) During PM specific attention has to be given to the presence of zoonotic agents such as mycobacterial infections. Traditionally the mandibular lymph nodes are cut and inspected. In risk based meat inspection MA serology is used (10,11,12).

Pig herds originating from different farms showed typical abscesses in the mesenteric lymph nodes, liver and spleen but sometimes also in the heart muscle, kidneys and lungs.

Risk factor analysis showed two possible routes of contamination; 1) contamination of the animal's drinking water and 2) contamination at the breeder farm because all piglets originated from the same breeder farm.

The objective of this study was to find out if an MA infection could be confirmed in the affected finishing pigs, on the finisher farms, on the breeder farm and in the sows. Additionally, this study also presents an evaluation of an alternative injection site for intradermal tuberculin testing.

Material and Methods

Breeder farm and finishing farms

The breeder farm, located in the South of the Netherlands, holds 3000 clinically healthy sows in group housing and produces approximately 85.000 piglets a year. The farm operates under an integrated farm assurance scheme (Dutch IKB) and has a high level of biosecurity control (eg. full site pest control by a third party, no outside access for the animals, only GMP+ certified feed is fed, all visitors must shower and dress in farm clothes before entering the farm etc.). The piglets are distributed around an age of 10 weeks to different fattening farms in Belgium.

The fattening farms are part of a cooperative group where all animals are fed from the same animal feed source. The different farms operate under an integrated farm assurance scheme (Belgian Codiplan plus) and have a high level of biosecurity control. The pigs are slaughtered at an age of 26 weeks in an abattoir in the Netherlands.

Environmental samples

On 2 finishing farms and the breeder farm environmental samples were collected. Drinking water, biofilm formation and debris in water and feed pipes, wood shavings and feed samples were collected.

Intradermal skin test

To screen the sow herd for a possible MA infection, 1000 newly arrived gilts, 1700 multiparous sows and 500 piglets aged 10 weeks or older were subjected to an intradermal tuberculin test at different visits within 2 months. The skin test was performed by administering 0.1 ml Avian Tuberculin PPD (25.000 I.U., ASG, Lelystad, The Netherlands) into the base of the ear using a McIntock pre-set syringe (Bar Knight, Glasgow, U.K). The reaction was read after 48 to 72 hours by inspecting the injection site for signs of swelling or redness.

To facilitate the skin testing procedure animals were restrained in individual pens making the injection site more accessible. To be able to assess if an alternative injection site would be effective we injected 900 animals both at the base of the ear as well as under the tail about 2 cm out of the median.

Blood sample and tissue sample collection at slaughter

Three consignments of sows (total n=66), all individually identified, were sampled at slaughter. During bleeding blood samples were collected in 10 ml test tubes for serum collection using a coagulation inducer. After coagulation samples were stored and shipped at 4°C to the laboratory. The laboratory carried out the MA-ELISA (PrioCHECK® M. avium Ab porcine).

A gross examination was carried out at PM giving special attention to both mandibular and mesenteric lymph nodes. Samples were collected and identified on the individual sow level to facilitate paired analysis.

Bacteriology and molecular detection by polymerase chain reaction

Samples were cultured as described by Komijn (1) and subsequently ZN positive colonies were further characterized at the species and subspecies level by real time polymerase chain reaction (PCR). Specific primers as described by Slana (13) for the detection of the specific insertion sequences IS1245, present in Mycobacterium avium subspecies avium (MAA) and Mycobacterium avium subspecies hominisuis (MAH) and IS901 present in MAA only, were used.

For the direct detection of MAA and/or MAH in tissue samples the same real time PCR was used as described above (13).

Results/discussion

Fattening pigs

In 15 deliveries from 4 different finishing farms lesions in different organs and lymph nodes were detected indicative of a mycobacterial infection. Prevalence of these abnormalities ranged from 0.5% up to 8.5% within a herd. Bacteriology was performed on affected tissue and revealed the presence of MAA (Table 1).

Sows

At the breeder farm the 500 piglets and the 1000 gilts tested negative in the tuberculin skin test. Of the 1700 sows 139 animals tested positive in the tuberculin skin test.

In 66 sows delivered to slaughter no gross pathological lesions were detected during PM inspection, even though 25 animals showed a positive skin test and 29 showed a positive result in serology (Table 2).

Comparison of ear and tail tuberculin skin testing.

In 900 animals tested both at the base of the ear and under the tail, 32 showed positive reactions in both tests, 4 only in the ear, 8 only under the tail and 856 tested negative (Table 3). This results in a very good agreement of both tests (Kappa= 0.835, CI

95% : 0.77-0.90 (14)) with good relative test characteristics for the alternative injection site compared to the base of the ear (SE=0.800, CI 95% : 0.68-0.92 SP=0.995, CI 95% : 0.989-0.999).

Environmental samples

Environmental samples on one of the finishing farms as well as on the breeder farm showed presence of generic *Mycobacteria*, but preliminary analysis could not confirm these to be MA.

Conclusion

For the purpose of screening large herds intradermal skin testing under the tail is an effective method compared to testing at the ear base. This facilitates rapid administration when the animals can only be restrained in a way where the head is presented away from the person carrying out the test.

No *Mycobacterium avium* could be recovered from the environmental samples or from the sows.

Results are indicative of an MA infection in the sows but this could not be proven conclusively at this stage. Further investigation is necessary.

If an infection in the sows can be demonstrated the sources of infection have to be identified to successfully control the spread of MAA within the farm.

This case study show that when information collected during post mortem inspection is fed back to the farm of origin, farmers and veterinarians can take corrective and preventive measures to control zoonotic infections and improve animal health.

References

1. Komijn, R.E., 1999, Prevalence of *Mycobacterium avium* in slaughter pigs in The Netherlands and comparison of IS1245 restriction fragment length polymorphism patterns of porcine and human isolates. *Journal of Clinical Microbiology* 1999, 37, 1254-1259.
2. Möbius, P., 2006, Comparative macrorestriction and RFLP analysis of *Mycobacterium avium* subsp. *avium* and *Mycobacterium avium* subsp. *hominissuis* isolates from man, pig, and cattle. *Veterinary Microbiology* 2006, 117, 284-291.
3. Biet F., 2005, Zoonotic aspects of *Mycobacterium bovis* and *Mycobacterium avium*-intracellulare complex (MAC). *Vet Res.* 2005 May-Jun;36(3):411-36.
4. Wagner D., 2004. Nontuberculous mycobacterial infections: a clinical review. *Infection.* 2004 Oct;32(5):257-70.
5. Matlova L., 2004. Impact of sawdust and wood shavings in bedding on pig tuberculous lesions in lymph nodes, and IS1245 RFLP analysis of *Mycobacterium avium* subsp. *hominissuis* of serotypes 6 and 8 isolated from pigs and environment. *Vet Microbiol.* 2004 Sep 8;102(3-4):227-36.
6. Matlova, L., 2005, Distribution of *Mycobacterium avium* complex isolates in tissue samples of pigs fed peat naturally contaminated with mycobacteria as a supplement. *Journal of Clinical Microbiology* 2005, 43, 1261-1268.
7. Trckova, M., 2005 Peat as a feed supplement for animals: a review. *Veterinarni Medicina* 2005, 50, 361-377
8. Fischer O, 2000, Findings of mycobacteria in insectivores and small rodents. *Folia Microbiol (Praha).* 2000;45(2):147-52.
9. Thoen, C.O., 2006. Tuberculosis. In *Diseases of Swine*, 9th ed.; Straw B.E., Zimmermann J.J., D'Allaire S., Taylor D.J.

Table 1. Results of Post Mortem meat inspection and bacteriology of finishing pigs suspected of an MA infection.

Finishing pigs					
Farm	Slaughtered	Detected at PM	Bacterial analysis	negative	positive
A	1300	53	25	9	16
B	1980	29	5	0	5
C	671	15	1*	0	1*
D	605	5	5	2	3

* *pooled sample of affected tissues of 5 pigs.*

Table 2. Results of tuberculin skin testing, PM inspection, serology and bacteriology of 66 sows from a farm suspected of an MA infection.

Sows			
Test	negative	positive	total
Tuberculin skin testing	41	25	66
Post mortem inspection	66	0	66
Serology	37	29	66
Bacteriology	inconclusive		66

Table 3. Results of tuberculin skin testing of animals both in the ear base and under the tail.

Test position		ear		total
		positive	negative	
under tail	positive	32	4	36
	negative	8	856	864
	total	40	860	900

Eds.; Blackwell Publishing: Iowa, USA, 2006; pp. 807-822.

10. Wisselink, H.J.; 2010. Serodiagnosis of *Mycobacterium avium* infections in pigs. *Veterinary Microbiology* 2010, 142, 401-407.
11. Hiller A, (2012) Implementation of risk based meat inspection of fattening pigs without incision in a slaughterhouse in Lower Saxony. *Archiv für Lebensmittelhygiene* 63: 4, 107-114
12. Hiller A.. (2013). Prevalence of *Mycobacterium avium* in slaughter pigs based on serological monitoring results. *Int. J. Environmental Research and Public Health*. Accepted.
13. Slana I, 2010 Distribution of *Mycobacterium avium* subsp. *avium* and *M. a. hominissuis* in artificially infected pigs studied by culture and IS901 and IS1245 quantitative real time PCR. *Vet Microbiol.* Aug 26;144(3-4):437-43.
14. Landis, J. R., & Koch, G. G. (1977). The measurement of observer agreement for categorical data. *biometrics*, 159-174.